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Patent

Attorney's Docket No. 000500-285
TECH CENTER 1600/2900

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
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Dan HAUZENBERGER) Group Art Unit: 1645
)
Application No.: 09/763,292) Examiner: Unassigned
)
Filed: February 21, 2001)
)
For: A METHOD FOR MEASURING A)
PATIENT'S ABILITY TO)
METABOLIZE CERTAIN DRUGS)
(as amended))
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SUPPLEMENTAL PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicant filed a Preliminary Amendment on February 21, 2001. In accordance with the new PTO rules and procedures, applicant provides herewith a clean set of claims, as well as a marked-up copy.

IN THE CLAIMS

Please replace claims 2-3 and 16-18, as follows.

2. (Amended) A method according to claim 1, wherein the single-stranded DNA isolated and/or provided in step a) is obtained by performing a modified amplification reaction in which one of the two amplification primers comprises a first attachment moiety bound to the primer, thereby obtaining a double-stranded amplification product in which only one of the strands comprises a first attachment moiety, where said first attachment

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moiety is one half of an affinity pair, and then simultaneously or sequentially in any order rendering the amplification product single-stranded and immobilising the strand comprising the first attachment moiety to a solid support with the aid of the other component of the affinity pair, whereafter all unbound material is removed.


3. (Amended) A method according to claim 1, wherein said point mutation to be detected only comprises one altered nucleotide.

16. (Amended) A kit for detecting a possible point mutation in cytochrome P450 isoform CYP2C19 comprising:

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- a) a detection primer according to claim 4;
 - b) two amplification primers derived from the sequence according to SEQ.ID.NO.1 and a sequence complementary to SEQ.ID.NO.1, said primers being chosen in such a way that a subsequence of the sequence according SEQ.ID.NO.1 containing said possible point mutation and a sequence complimentary to said detection primer can be amplified;
 - c) at least one labelled nucleoside triphosphate; and
 - d) a DNA polymerasing agent.

17. (Amended) A kit for detecting a possible point mutation in cytochrom P450 isoform CYP2C9 comprising:

- a) a detection primer according to claim 10;

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- b) two amplification primers derived from the sequence according to SEQ.ID.NO.2 and a sequence complementary to SEQ.ID.NO.2, said primers being chosen in such a way that a subsequence of the sequence according SEQ.ID.NO.2 containing said possible point mutation and a sequence complimentary to said detection primer can be amplified;
 - c) at least one labelled nucleoside triphosphate; and
 - d) a DNA polymerasing agent.

18. (Amended) A kit for detecting a possible point mutation in cytochrome P450 isoform CYP2C9 comprising:

- a) a detection primer according to claim 13;
 - b) two amplification primers derived from the sequence according to SEQ.ID.NO.3 and a sequence complementary to SEQ.ID.NO.3, said primers being chosen in such a way that a subsequence of the sequence according SEQ.ID.NO.3 containing said possible point mutation and a sequence complimentary to said detection primer can be amplified;
 - c) at least one labelled nucleoside triphosphate; and
 - d) a DNA polymerasing agent.
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Amended claims

1. A method for determining the ability of cells in a sample, to metabolise a certain drug, comprising the steps of
- 5 a) isolating and/or providing detectable amounts of single-stranded DNA from said sample by using known methods;
- b) hybridising the single-stranded DNA obtained in step a) with a detection primer comprising a plurality of nucleotide residues, said primer being complementary to a target nucleotide sequence immediately adjacent and 5' in relation to a defined point mutation of a single-stranded DNA encoding a cytochrome P450 isoform, such as i) mutations resulting in an amino acid substitution at amino acid residue 144 of CYP2C9*2; ii) mutations resulting in an amino acid substitution at amino acid number 359; iii) a point mutation at base 686 of a nucleic acid sequence encoding the M1 allele of CYP2C19; and iv) a point mutation at base 641 of a nucleic acid sequence encoding the M2 allele of CYP2C19, where said point mutation is known to affect said isoform's ability to metabolise said drug, when the detection primer is hybridised to the target nucleic acid;
- 10 c) extending the primer using a polymerising agent in a mixture comprising one or more nucleoside triphosphates wherein the mixture includes at least one labelled nucleoside triphosphate complementary to either the first or second nucleic residue, and optionally one or more chain terminating nucleoside triphosphates;
- 15 d) detecting the incorporation of the nucleoside triphosphate using said means, whereby it is determined whether said sample contains said point mutation of said cytochrome P450 isoform.
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- 25 *Sub 1* 2. A method according to claim 1, *wherein* ~~characterised in that~~ the single-stranded DNA isolated and/or provided in step a) is obtained by performing a modified amplification reaction in which one of the two amplification primers comprises a first attachment moiety bound to the primer, thereby obtaining a double-stranded amplification product in which only one of the strands comprises a first attachment moiety, where said first attachment moiety is one half of an affinity pair,
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and then simultaneously or sequentially in any order rendering the amplification product single-stranded and immobilising the strand comprising the first attachment moiety to a solid support with the aid of the other component of the affinity pair, whereafter all unbound material is removed.

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3. A method according to ~~any one of claim 1 or claim 2~~ ^{claim 1, wherein}, characterised in that said point mutation to be detected only comprises one altered nucleotide.

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4. A detection primer hybridising to a target nucleotide sequence immediately adjacent and 5' in relation to a point mutation of a single stranded DNA encoding cytochrome P450 isoform CYP2C19, said primer consisting of a subsequence of 10 - 70 nucleotides of the sequence:

5' GTTCTTTTAC TTTCTCCAAA ATATCACTTT CCATAAAAGC
AAGGTTTTTA

15 AGTAATTGT TATGGCTTCC 3'

which subsequence always comprises the nucleotide located in the 3' end of the sequence above.

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5. A detection primer according to claim 4 consisting of a subsequence of 10-30 nucleotides.

6. A detection primer according to claim 5 which is 5'-
AAGTAATTGTATGGGTCC-3'.

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7. A detection primer hybridising to a target nucleotide sequence immediately adjacent and 5' in relation to a point mutation of a single stranded DNA encoding cytochrome P450 isoform CYP2C19, said primer consisting of a subsequence of 10 - 70 nucleotides of the sequence:

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5'-TTGAATGAAA ACATCAGGAT TGTAAGCACC CCCTGA-
ATCC AGATATGCAA

TAATTTTCCC ACTATCATTG ATTATTTCCC-3'

which subsequence always comprises the nucleotide located in the 3' end of the sequence above.

8. A detection primer hybridising to a target nucleotide sequence immediately adjacent and 5' in relation to a point mutation of a single-stranded DNA encoding cytochrome P450 isoform CYP2C19, said primer consisting of a subsequence of 8 - 50 nucleotides of the sequence:

5'-AACTTGATGG AAAAATTGAA TGAAAACATC AGGATTG-TAA GCACCCCCTG-3'

which subsequence always comprises the nucleotide located in the 3' end of the sequence above.

9. A detection primer according to claim 8 which is: 5'-GATTGTAAGCACCCCCTG-3'.

10. A detection primer hybridising to a target nucleotide sequence immediately adjacent and 5' in relation to a point mutation of a single-stranded DNA encoding cytochrome P450 isoform CYP2C9, said primer consisting of a subsequence of 8 - 50 nucleotides of the sequence:

5'-CCCTCATGAC GCTGCGGAAT TTTGGGATGG GGAAGAG-GAG CATTGAGGAC-3'

which subsequence always comprises the nucleotide located in the 3' end of the sequence above.

11. A detection primer according to claim 10 which is: 5'-AAGAGGAGCATTGAGGAC-3'.

12. A detection primer hybridising to a target nucleotide sequence immediately adjacent and 5' in relation to a point mutation of a single-stranded DNA encoding cytochrome P450 isoform CYP2C9, said primer consisting of a subsequence of 8 - 50 nucleotides of the sequence: